

REMARKS

The Office Action and the cited and applied references have been carefully reviewed. No claim is allowed. Nonelected claims 3-6 are cancelled without prejudice to the filing of a divisional application thereon. Claims 1 and 2 presently appear in this application and define patentable subject matter warranting their allowance. Reconsideration and allowance are hereby respectfully solicited.

Claims 1 and 2 have been rejected under 35 U.S.C. §112, first paragraph, because the examiner states that the specification, while being enabling for two hyperthermostable protease enzymes comprising the amino acid sequence of SEQ ID NO:1 or 5, does not reasonably provide enablement for any functional equivalents (i.e., mutants and variants having the very same function of being a hyperthermostable protease). This rejection is respectfully traversed.

Claims 1 and 2 are amended to now recite that the functional equivalent of the hyperthermostable protease has the same amino acid sequence as SEQ ID NO:1 or 5, but with the exception that one amino acid residue to 5% of the amino acid residues of SEQ ID NO:1 or 5 are mutated. SEQ ID NO:5 is a hybrid hyperthermostable protease containing sequences from PFUS protease (SEQ ID NO:3) and TCES protease (SEQ ID NO:1).

A BLAST amino acid sequence alignment of SEQ ID NO:1 (TCES protease designated as sequence 1 in the alignment) and SEQ ID NO:3 (PFUS protease designated as sequence 2 in the alignment) disclosed in the instant application is attached hereto for the examiner's consideration. The calculated sequence homology is 78%, with homologous but not identical amino acid residues (i.e., Ile and Val) at particular positions indicated by the symbol "+" in the line between the aligned sequences. Furthermore, the specification at page 20, lines 8-17, teaches that there is extremely high homology around four amino acid residues (citing Protein Engineering, Volume 4, pages 719-737 (1991)), which are considered to be important for catalytic activity of the protease (see also Figures 11 and 12 of the instant application). These amino acid residues are:

SEQ ID NO:1	167D, 200H, 299N, 362S
SEQ ID NO:3	34D, 67H, 167N, 230S
SEQ ID NO:5	166D, 199H, 299N, 362S

as would be readily recognized and understood by one of skill in the art. This same person of skill in the art would furthermore understand that because of the importance of these residues for protease catalytic activity, it would be desirable not to introduce changes to these residues or to neighboring amino acid sequences. However, changes such as

substitution with conservative amino acid residues can safely be made of positions indicated by the symbol "+". In addition, amino acid substitutions of residue positions which are not conserved or homologous can also be made. Accordingly, one of skill in the art, based on the above information and the guidance in the specification, including the protease activity assay at pages 50-53 and the thermostability assay at pages 58-61, is fully enabled to make functional equivalents of the hyperthermostable proteases of SEQ ID NO:1 or 5 in which one residue up to 5% of the amino acid residues are mutated.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 1-2 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is respectfully traversed.

Claims 1 and 2 are now amended to recite that the functional equivalents of the hyperthermostable protease of SEQ ID NO:1 or 5 are those in which a single amino acid residue up to 5% of the amino acid residues are changed. The

USPTO's "Synopsis of Application of Written Description Guidelines" provides Example 14 (Product by Function) which teaches what would be required to satisfy the written description requirement for variants of a protein/enzyme, which variants have at least 95% sequence identity to SEQ ID NO:3 and catalyze the reaction of A to B. The analysis for this example teaches that the single disclosed species of SEQ ID NO:3 is representative of the genus because all members have at least 95% structured identify with the reference compound (SEQ ID NO:3) and because of the presence of an assay which applicant provided for identifying all of the at least 95% identified variants of SEQ ID NO:3 which are capable of the specified catalytic activity. The analysis of Example 14 concludes by stating the disclosure meets the written description requirements for a protein having SEQ ID NO:3 and variants thereof that are at least 95% identical to SEQ ID NO:3 and catalyze the reaction of A to B.

The presently claimed functional equivalent of a hyperthermostable protease has at least 95% sequence homology/identity to the hyperthermostable protease of SEQ ID NO:1 or 5, and the enzymatic activity and thermostability property can be readily assayed as disclosed in the specification at pages 50-53 and 58-61. Accordingly, the functional equivalents recited in the claims are adequately

described in the present specification and therefore satisfy the written description requirement.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 1-2 have been rejected under 35 U.S.C. §102(b) as being anticipated by Ilse et al. (Blumenthal et al.), *Appl. Environ. Microbiol.*, 56(7):1992-1998 (1994) or Morikowa et al., *Appl. Environ. Microbiol.*, 60(12):4559-1998 (1994). This rejection is respectfully traversed.

From the examiner's comments, the examiner appears to be indicating that it is only the functional equivalents recited in the present claims that are taken as reading on the enzymes of Ilse and Morikowa because there is no limitation placed on the number of changes that can be present in the polypeptide sequence of SEQ ID NO:1 or 5. Claims 1 and 2 are now amended to positively recite that, in the functional equivalents, there are only from a single residue to at most 5% of the amino acid residues that are changed from the sequences of SEQ ID NO:1 or 5. Accordingly, Ilse (Blumenthal) or Morikowa can not anticipate the presently claimed hyperthermostable protease of SEQ ID NO:1 or 5 or the recited functional equivalents thereof.

Furthermore, the presently claimed hyperthermostable protease is believed to be a serine protease because it is

inhibited by a typical serine protease inhibitor, PMSF, as disclosed on page 65, line 21, to page 67, line 20 and Table 1 of the present specification. By contrast, Morikowa's enzyme is a thiol protease which is not inhibited by PMSF as clearly shown in Table 2 on page 4564.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 1-2 have been rejected under 35 U.S.C. §101 as claiming the same invention as that of claim 1 of prior US Patent No. 6,261,822. The examiner states that this is a double patenting rejection and takes the position that the hyperthermostable protease of SEQ ID NO:3 recited in claim 1 of '822 is a functional equivalent of either SEQ ID NO:1 or 5.

This rejection is obviated by the amendments to claims 1 and 2. As discussed above and as can be seen from the amino acid sequence alignments of SEQ ID NOs:1 and 3 attached hereto, the % sequence identity between SEQ ID NO:1 and 3 is 78%. This means that claim 1 of '822 cannot anticipate the functional equivalents of hyperthermostable protease of SEQ ID NO:1 in which only a single amino acid residue up to 5% of the amino acid residues are changed.

With regard to the hyperthermostable protease of SEQ ID NO:5, which is hybrid of SEQ ID NOs:1 and 3, where residues 133-261 of SEQ ID NO:5 are residues 1-129 of SEQ ID NO:3 and

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Reply to Office Action of January 19, 2007

residues 262-659 of SEQ ID NO:5 are residues 262-659 of SEQ ID NO:1. Accordingly, claim 1 of '822 also cannot anticipate the recited functional equivalents of the hyperthermostable protease of SEQ ID NO:5 because there is much more than a 5% difference in sequence identity between SEQ ID NOs:3 and 5.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

In view of the above, the claims comply with 35 U.S.C. §112 and define patentable subject matter warranting their allowance. Favorable consideration and early allowance are earnestly urged.

Respectfully submitted,

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# Blast 2 Sequences results

PubMed

Entrez

BLAST

OMIM

Taxonomy

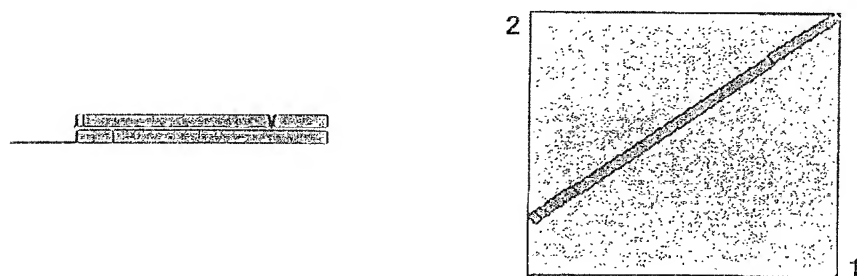
Structure

## BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.15 [Oct-15-2006]

Matrix:  gap open:  gap extension:   
 x\_dropoff:  expect:  wordsize:  Filter ☐ View option:   
 Masking character option:  Masking color option:   
☐ Show CDS translation

Sequence 1:  SEQ 3  
 Length = 522 (1 .. 522)

Sequence 2:  SEQ 1  
 Length = 659 (1 .. 659)



NOTE: Bitscore and expect value are calculated based on the size of the nr database.

Score = 825 bits (2132), Expect = 0.0  
 Identities = 407/520 (78%), Positives = 443/520 (85%), Gaps = 7/520 (1%)

Query	9	SAAQVMATYVWN-LGYDGSGITIGI IDTGIDASHPDLQGKVI GWDFVNGRSYPYDDHGH	67
		S +Q+ A VWN LGYDGS+ + I+DTGIDA+HPDL+GKVICW D VNGRS PYDD GH	
Sbjct	141	SVSQICADTVWNSLGYDGSVVVAIVDTGIDANHPDLKGVIGWYDAVNGRSTPYDDQGH	200
Query	68	GTHVASIAAGTGAASNGKYKGMAPGAKLAGIKVLGADGSGSISTIIKGVEWAVDNKDKYG	127
		GTHVA I AGTG+ N +Y G+APGAKL G+KVLGADGSGS+STII GV+W V NKDKYG	
Sbjct	201	GTHVAGIVAGTGSV-NSQYIGVAPGAKLVGVKVLGADGSGSVSTIIAGVDWVVQNKDKYG	259
Query	128	IKVINLSLGSQSSDGTDAALSQAVNAWDAGLVVVVAAGNSGPNKYTTIGSPAAASKVITV	187
		I+VINLSLGSQSSDGTDL+SQAVN AWDAG+VV VAAGNSGPN YT+GSPAAASKVITV	
Sbjct	260	IRVINLSLGSQSSDGTDSLSQAVNNAWDAGIVVCVAAGNSGPNYTTVCGSPAAASKVITV	319
Query	188	GAVDKYDVITSFSSRGPTADGRLKPEVVAPGNWIIAARASGTSMGQPINDYYTAAPGTSM	247
		GAVD D I SFSSRGPTADGRLKPEVVAPG IIA RASGTSMG PINDYYT A GTSM	
Sbjct	320	GAVDSNDNIASFSSRGPTADGRLKPEVVAPGVDIIAPRASGTSMGTPINDYYTKASGTSM	379
Query	248	ATPHVAGIAALLLQAHPSWTPDKVKKTAL IETADIVKPDEIADIAYGAGRVNAYKAINYDN	307
		ATPHV+G+ AL+LQAHPSWTPDKVKKTAL IETADIV P EIADIAYGAGRVN YKAI YD+	
Sbjct	380	ATPHVSGVGALILQAHPSWTPDKVKKTAL IETADIVAPKEIADIAYGAGRVNVYKAIKYDD	439
Query	308	YAKLVFTGYVANKGSQTHQFVLSGASEVTATLYWDNANSOLDLYLYDPNGNQVDYSYTAY	367
		YAKL FTG VA+KGS TH F +SGA+FTVATLYWD +SD+DLLYLYDPNGN+VDYSYTAY	
Sbjct	440	YAKLTFTGVSADKGSATHTFDVSGATFVTATLYWDTGSSDIDLYLYDPNGNEVDYSYTAY	499



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Query 368 YGFEKVGYYNPTDGTWTIKVVSYSGSANYQVDVSDGSLSQ-----PGSSPSPQPEPTVD 422
          YGFEKVGYYNPT GTWT+KVVSY G+ANYQVDVSDGSLSQ P +P+P P PT D
Sbjct 500 YGFEKVGYYNPTAGTWTVKVVSYKGAANYQVDVSDGSLSQSGGNNPNNPNPNPTPTTD 559

Query 423 AKTFQXSDHYYYDRSDFTMTVNSGATKITGDLVFDTSYHDLDLYLDPNQKLVDRSESP 482
          +TF S + Y+D SDFTM VNSGATKITGDL FDTSY+DLDLYLDPN LVDRS S
Sbjct 560 TQTFTGSVNDYWDTSDFMTMNVNSGATKITGDLTFDTSYNDLDLYLDPNGNLVDRSTSS 619

Query 483 NSYEHVEYLTAPGTWYFLVYAYTYGWAYYELTAKVYYG 522
          NSYEHVEY PAPGTW FLVYAY TYGWA Y+L A VYYG
Sbjct 620 NSYEHVEYANPAPGTWTFVYAYSTYGWADYQLKAVVYYG 659
```

CPU time: 0.03 user secs. 0.00 sys. secs 0.03 total secs.

Lambda	K	H
0.313	0.132	0.395

Gapped Lambda	K	H
0.267	0.0410	0.140

#### Matrix: BLOSUM62

Gap Penalties: Existence: 11, Extension: 1

Number of Sequences: 1

Number of Hits to DB: 3575

Number of extensions: 2009

Number of successful extensions: 7

Number of sequences better than 10.0: 1

Number of HSP's gapped: 1

Number of HSP's successfully gapped: 1

Length of query: 522

Length of database: 1,660,314,297

Length adjustment: 140

Effective length of query: 382

Effective length of database: 1,660,314,157

Effective search space: 634240007974

Effective search space used: 634240007974

Neighboring words threshold: 9

X1: 16 (7.2 bits)

X2: 129 (49.7 bits)

X3: 9 (49.7 bits)

S1: 42 (21.9 bits)

S2: 82 (36.2 bits)